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Article

Building-Level Wastewater Surveillance for SARS-CoV-2 in Occupied University Dormitories as an Outbreak Forecasting Tool: One Year Case Study

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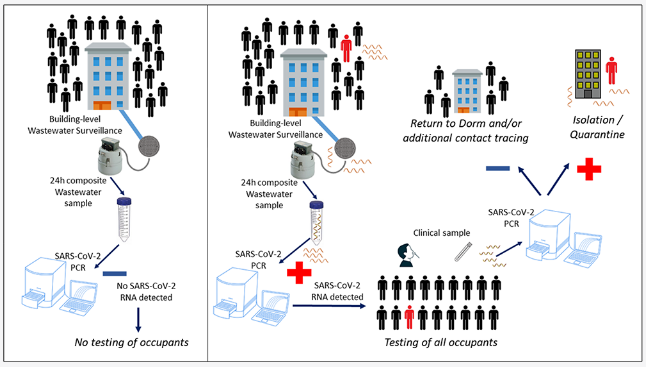
ABSTRACT: Congregate living poses one of the highest risk situations for the transmission of respiratory viruses including SARS-CoV-2. University dormitories exemplify such high-risk settings. We demonstrate the value of using building-level SARS-CoV-2 wastewater surveillance as an early warning system to inform when prevalence testing of all building occupants is warranted. Coordinated daily testing of composite wastewater samples and clinical testing in dormitories was used to prompt the screening of otherwise unrecognized infected occupants. We overlay the detection patterns in the context of regular scheduled occupant testing to validate a wastewater detection model. The trend of wastewater positivity largely aligned well with the clinical positivity and epidemiology of dormitory occupants. However, the predictive ability of wastewater-surveillance to detect new positive cases is hampered by convalescent shedding in recovered/noncontagious individuals as they return to the building. Building-level pooled wastewater-surveillance and forecasting is most productive for predicting new cases in low-prevalence instances at the community level. For higher-education facilities and other congregate living settings to remain in operation during a pandemic, a thorough surveillance-based decision-making system is vital. Building-level wastewater monitoring on a daily basis paired with regular testing of individual dormitory occupants is an effective and efficient approach for mitigating outbreaks on university campuses.



KEYWORDS: *wastewater surveillance, COVID-19, SARS-CoV-2, university housing, composite sample*

■ INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly contagious respiratory virus that has caused numerous large outbreaks in congregate living settings since the start of the pandemic.[1] Like many respiratory viruses, several mitigating factors such as nonpharmaceutical interventions to prevent transmission (masking, avoidance of close contact, and negative air pressure rooms) are not feasible in many places where individuals reside together. Congregate living settings such as nursing homes, university dormitories, barracks, and prisons can all facilitate viral transmission. In addition and somewhat specific to SARS CoV-2 is a substantial asymptomatic and presymptomatic shedding phase wherein the infected person does not show symptoms, so that they do not get tested or self-isolate to prevent further spread.[2,3] This can contribute to efficient secondary spread in congregate living settings.

Fecal SARS-CoV-2 RNA shedding is commonly detected in patients with COVID-19, which makes it of interest to use wastewater-surveillance as a way to understand the presence and scale of infection.[4] Wastewater-surveillance of SARS-CoV-2 employs reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) for detecting unique SARS-CoV-2 genes on the viral RNA in wastewater samples.[5−8] Wastewater-surveillance at community scale provides early indication of COVID-19 prevalence; however, it is not particularly valuable for identifying point-source of positive individuals, narrowing down cases to a specific location, making decisions for targeted prevalence testing, and/or implementing interventions to contain transmissions. A more compartmentalized, building-level approach can help circumvent this challenge via devising a close-to-source wastewater-surveillance at congregate living settings. This relatively new but strikingly beneficial approach

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has drawn the attention of several universities, within the U.S. and abroad.[9−12] As per a recent review, over 200 university and college campuses in the U.S. implemented pooled wastewater-surveillance during the fall 2020 semester.[9] Such building-level wastewater monitoring paired with prevalence testing can assist in making data-driven decisions for the management of testing and isolation and even identification of unrecognized cases before widespread transmissions can occur. Nonetheless, to be effective and draw conclusive interpretations require a well-coordinated effort that includes practical application of the data on the logistic capabilities of the situation.

While vaccination efforts and potential treatments are progressing, asymptomatic testing of individuals at risk for transmission is critical.[13] Early in the pandemic prior to widespread vaccine availability and testing, there was a need for surveillance in congregate living situations as an early warning system, although the approach was not uniform.[9,10,12] We aimed to evaluate the predictive value of wastewater testing in two specific contexts in a largely unvaccinated congregate living population (with and without standing weekly asymptomatic surveillance testing of individuals). Due to the lack of comprehensive testing available early in the fall 2020 semester, wastewater- surveillance was employed to prompt point prevalence testing of all dormitory occupants in specific monitored buildings. In the spring semester, all previously uninfected dormitory occupants were tested weekly using a saliva-based test. This provided an opportunity to assess the difference in testing strategy as well as the utility of wastewater in the two different contexts.

We initially used validated wastewater collection, concentration, and molecular methods within our campus and hospital[14] prior to a larger return of students. In the current study, we examined the utility and further validated the performance of daily pooled wastewater surveillance of SARS-CoV-2 in conjunction with individual testing of dormitory residents for outbreak mitigation on a university campus. Three specific objectives of the current study were as follows: (i) implement widespread, frequent building-level wastewater surveillance of SARS-CoV-2 in occupied dormitories as a passive monitoring tool to mitigate outbreaks, (ii) develop an approach to interpret and use wastewater surveillance results as part of an integrated decision-support system, and (iii) highlight practical and contextual factors (i.e., "lessons learned") influencing the interpretation and usefulness of building-level pooled wastewater-surveillance results. Notably, the time period of this study (fall 2020−spring 2021) encompasses dramatically different conditions. For fall 2020, all students were unvaccinated, and they were not subject to individual prevalence testing. For spring 2021, students were mostly unvaccinated, but they were subject to weekly prevalence testing in addition to pooled, wastewater-based surveillance.

METHODS

University Setting. This study was performed at the University of Virginia, Charlottesville, a state public university consisting of 12 schools with an affiliate health system, which enrolls approximately 25 000 students in an academic year (70% undergraduate and 30% graduate). The university Department of Student Health and Wellness (SHW) is a fully accredited healthcare facility and the primary outpatient medical clinic for the university student population. SHW was responsible for all medical care, testing, and support for students experiencing COVID-19 symptoms, exposure, or disease among students.

The testing reported in this study was performed between Sept−Nov 2020 and Jan−May 2021, coinciding with the fall 2020 and spring 2021 academic semesters, respectively, at the University of Virginia, Charlottesville.

Individual Clinical Testing. All occupants were required to submit a prearrival COVID nucleic acid test prior to their arrival on campus. Testing of the occupants across both semesters included ready access to RT-PCR-based symptomatic nasopharyngeal (NP) testing (Abbott Alinity, Abbott m2000 Chicago, IL) through SHW and analyzed at the University of Virginia Health System's Medical Laboratories. During the fall semester, scheduled testing of asymptomatic students in a particular dormitory (or point prevalence testing) was performed with NP swabs processed as above with students directed to shelter in place until results returned. Subsequently, a location for observation of self-collected midturbinate swabs for symptomatic persons were run via the medical laboratories using the same platforms as the SHW testing. Lastly, a saliva-based testing laboratory was established for standing asymptomatic testing using TaqPath COVID-19 (Thermo Fisher, Waltham, MA). The saliva-based testing was expanded for spring semester where all students were tested weekly. Students who lived in the dormitories were monitored for compliance and expected to test with their dormitory as above with the following exemption: testing was not required for students 90 and 120 days following a positive result during the fall and spring semester, respectively. Antigen testing was not used in this specific university campus strategy.

Isolation and Quarantine. A broad, interdisciplinary isolation and quarantine team was created with representatives from across the university to serve students who required isolation and/or quarantine due to COVID-19 symptoms, exposure, or disease.[15] The team identified housing spaces among dormitories, apartments, and local private hotels for students who required isolation and quarantine.

Occupants who tested positive were assigned to isolation housing on the date the positive test result was received and remained there for at least 10 days from symptom onset or positive test. They were discharged only if they were fever-free for 24 h and their symptoms had improved or resolved, per CDC guidelines.[16] Occupants who were exposed to a COVID-19 positive case or had COVID-19 symptoms were placed into quarantine or person under investigation (PUI) housing immediately and tested 5−7 days postexposure if asymptomatic or as soon as feasible if symptomatic. Occupants who tested positive were placed in isolation for 10 days from symptom onset or positive test result and followed the same discharge process as outlined above for positive cases.[15] Occupants who tested negative and had a close contact exposure remained in quarantine for 14 days from the last date of exposure (Figure S1).

Data Management of Clinical Cases. The Student Health Research Database (SHRD) is an IRB-approved database (IRB#21090, 21255), which links multiple distinct academic and clinical data sets across the university including SHW's electronic medical record system (Medicat), the university's student registry database (Student Information System (SIS)), the Dean of Student's incident management system database (Safe Grounds (SG)), and the University Health System's electronic medical record warehouse (Data Warehouse).

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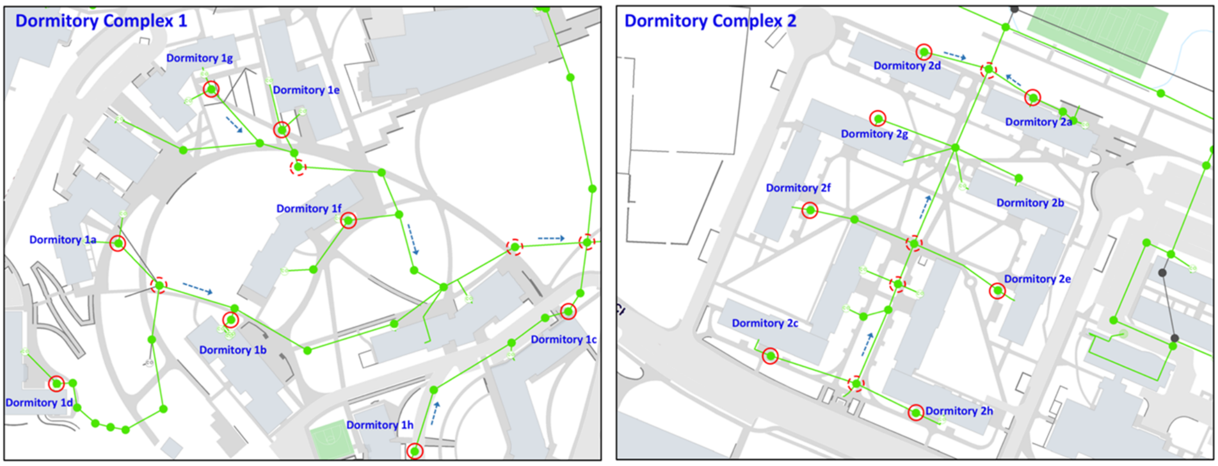

Figure 1. Layout of the Dormitory housing complexes sampled for wastewater. Green line represents wastewater drainage network, green circles the sewer maintenance hole access-points, red solid circles mark the sewer maintenance holes sampled at building-level and red dashed circles mark the subcommunity sewer maintenance holes (merging wastewater from two or more dormitory buildings) sampled within each dormitory complex. Direction of the wastewater flow is depicted using dashed arrows.

A multistep process was employed to link all the databases of interest within SHRD. First, the rosters of all students living in on-campus dormitories was obtained and linked to the COVID-19 testing results from the Data Warehouse using a unique identifier. Next, the data were linked to the SG database to collect information on isolation start dates and end dates (i.e., when the students were taken out of the dormitory and when they returned 10 days after isolation and 14 days after quarantine). Tests performed outside of the university were linked using COVID-19 specific templates created within Medicat (SHW Electronic Medical Record, Atlanta, GA) and used by licensed medical providers at SHW.

Duplicate information, such as in the case when a student had multiple positive COVID-19 test results, were examined manually with only the date of the first positive test (within 90 days for fall 2020 or 120 days for spring 2021) recorded in the finalized data set. This was done to avoid mis-categorizing a convalescent shedding individual as a new positive case. Personal identifiers were then removed and a comprehensive, integrated, deidentified data set was obtained, for use in comparing with the corresponding wastewater sampling results. The research team did not have access to individual charts for dormitory occupants but was instead provided testing results in aggregate form by building.

Wastewater Sampling Locations. Prior to the beginning of the fall 2020 academic semester, a wastewater sampling strategy was devised. This strategy sought to optimize use of limited autosampler (AS) equipment. Factors considered in the wastewater sampling and allocation of AS included: the layout and style of specific campus dormitories (e.g., hall- vs apartment-style), dormitory occupancy, student move-in information, layout of the wastewater collection system (sanitary distribution map), physical location, and accessibility of the sewer maintenance holes. On the basis of experience from the pilot wastewater testing surveillance initiative[14] and experiences reported by others,[10,11] composite wastewater samples were collected over a 20−22 h period (typically starting at 9−10 AM and operating through 6−7 AM the following day for collection) using commercially available AS.

Due to the very limited number of AS available (n = 5) in fall 2020, preliminary sampling was done relatively large groups of individuals, focusing on access point sewer maintenance holes downstream from multiple dormitories (Figure 1). Subsequently as more ASs were procured, testing protocols were optimized, and the daily work-flow was revised, the number of sampling locations was expanded, and samples were collected at individual building level. Dormitory complexes 1 and 2 had occupancies of 1166 and 850, respectively, during fall 2020. These numbers increased to 1323 and 958, respectively, during spring 2021. Building occupancy where wastewater was tested ranged from 97 to 181 residents per dormitory in fall 2020 and 107−200 residents per dormitory in spring 2021. These dormitories were selected as they all had a hall-style layout, double occupancy rooms, ∼45 occupants per floor and shared bathrooms (in contrast to apartment-style dormitories). It was presumed that occupants in these buildings were at a higher risk for outbreak than individual living in other kinds of dormitories. Although a total of 30 unique sewer maintenance holes were sampled across 9 dormitory complexes on the University premises, data from 16 dormitories (8 each in Complexes 1 and 2) that were most consistently surveilled via wastewater sampling was considered for this analysis. Occupancy details of the 16 dormitories are presented in Table 1. Sampling sites were prioritized on the basis of resident occupancy, building layout, proximity of the buildings in the dormitory complex and exclusiveness of the sewer maintenance hole in obtaining wastewater discharge from a building.

Wastewater Sample Collection. As described previously,[14] AS950 (HACH, Loveland, CO) and GLS compact (TELEDYNE ISCO, Lincoln *NE*) autosamplers (AS) were used for collection of composite wastewater samples (Figure S2a,b).

Due to the shortage and delay in procuring additional AS from manufacturers, and to address the urgency in the need for building-level wastewater surveillance, the University of Virginia Facilities Management team built homemade UVA-AS (Figure S2c) on the basis of instructions provided by Syracuse University with some modifications.[17] The ASs were programmed to draw 30 mL of wastewater at 15 min intervals. Composite ranging from 16 to 26 h samples were collected on ice. Each day during wastewater sample retrieval, AS program end-time, missed sampling events, wastewater volume collected in the jar, and errors if any were systematically

Table 1. Sampling Sites and Corresponding Resident Occupancy at Move-In

	Dormitory Complex	fall 2020 occupancy	spring 2021 occupancy
	Dormitory Complex 1	1166	1323
1	Dormitory 1 a	176	190
2	Dormitory 1 b	101	120
3	Dormitory 1 c	181	200
4	Dormitory 1 d	152	172
5	Dormitory 1 e	134	162
6	Dormitory 1 f	147	170
7	Dormitory 1 g	174	184
8	Dormitory 1 h	101	125
	Dormitory Complex 2	850	958
9	Dormitory 2 a	97	107
10	Dormitory 2 b	104	119
11	Dormitory 2 c	109	127
12	Dormitory 2 d	106	120
13	Dormitory 2 e	118	127
14	Dormitory 2 f	105	121
15	Dormitory 2 g	104	125
16	Dormitory 2 h	107	112
	total	2016	2281

logged onsite. Aliquot of composite samples in replicates were immediately transported to a laboratory and processed the same day. After recharging AS batteries, emptying leftover wastewater in the collection jars, cleaning, and ensuring the strainer connected to the suction line was positioned aligning wastewater stream, AS programs were restarted for next day's collection.

Wastewater Concentration. An ultracentrifugation method as described previously[14] was employed for the concentration of wastewater samples. The composite wastewater sample (40 mL) was transferred into an ultracentrifuge test tube (Beckman Coulter, Indianapolis, IN), and 24 mL of 50% sucrose in TNE buffer was carefully pipetted below the wastewater sample. Samples were subsequently centrifuged at 42 000 rpm (~150 000*g*) for 45 min in a Beckman Coulter LE80 Ultracentrifuge. The supernatant was decanted and the pellet was suspended in 300 μL of PBS solution for subsequent processing.

Molecular Methods (RNA extractions and PCR). The resuspended pellet samples were processed for RNA extraction using NucleoSpin RNA Plus Kit (Takara Bio USA Inc., Mountain View, CA) following the manufacturer's protocol. PCR assays were run on an ABI 7500 Fast Real Time PCR system (Applied Biosciences, Foster City, CA) using primers and methods to amplify RNaseP (Rp) (PCR human control), N1, and N2 SARS-CoV-2 viral targets, as specified in the CDC protocol for SARS-CoV-2 analysis (https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html). The RP internal control is a human fecal indicator, which was used to confirm sewage presence and rule of possible sample inhibition. Cycle threshold (Ct) values for each of three target genes from the PCR assay was recorded for subsequent analyses. All runs included positive, negative, and water blank controls. The same set of samples were initially run as duplicates during the validation phase[14] but only run as a single sample for this study. The criterion of result was adapted from the cutoff for clinical diagnostic testing (Ct values for N1 and N2 $\leq$ 40 were considered positive, clinical cutoff $\leq$45). Negative samples corresponded to Ct values >40 for N1 and N2, with RP $\leq$ 45. Results were indeterminate when only one of N1 and N2 target was positive. Samples exhibiting RP > 45 were considered failed. This was not run as a quantitative assay, and the limit of detection was determined in the previous study on the basis of number of positive and negative occupants in a building[14] but not using a known dilution from a standard for PCR. However, for the semiquantification of SARS-CoV-2 in wastewater, positive samples were further stratified into three groups on the basis of the lower Ct, namely, Ct = 40−35, Ct = 35−30, and Ct < 30 if the RP gene was detected in a noninhibited sample.

Data Analysis. We overlaid the SARS-CoV-2 RNA signals obtained from wastewater samples-Ct values, atop the epidemiological data (i.e., the number of positives cases) for the same day. In case of wastewater, the lower Ct value for N1 and N2 genes obtained for each sample was considered. For clinical cases, we deduced that the seven day moving average was computed centered at the date (3 days before − 3 days after).

To further understand the impact of prolonged convalescent shedding on wastewater positivity from an occupied building, the number of shedders present in the dormitory building and Ct score were overlaid. The Ct score is a rough estimate of the viral load in the wastewater, on the basis of an empirical calibration[10] (eq 1). This equation was used to estimate viral load over time on the basis of Ct measurements from sampled buildings. N is the number of people in the dormitory during sampling. VC is the virus concentration (genome copies/L) in the wastewater sample.

$$\mathrm{Ct} = 40 - 3 \times \log_{10}\left(\frac{\mathrm{VC}}{N}\right) \tag{1}$$

Specifically, $\log_{10}(\mathrm{VC}/100)$ was used as our Ct score to impute an estimated value of viral load per day in a specific building, based on daily measurement of wastewater Ct value because we were not using a quantitative PCR method.

RESULTS

Challenges in Early Fall 2020. Wastewater sample collection spanned 12 continuous weeks in fall 2020 (Aug 26, 2020 to Nov 20, 2020), with a total of 63 sampling days. Wastewater sampling time frame in spring 2021, was 14 continuous weeks (Jan 2, 2021 to May 3, 2021) spanning 67 sample days. Wastewater sampling and testing was performed at 5 d/week frequency (occasionally at 6−7 d/week when for some portions of each semester when case-counts were particularly high). With respect to sampling strategy, the first few weeks in fall 2020 were a learning phase and wastewater results quality was suboptimal, because there were too few AS to get good coverage across all dormitories, and the sample collection workflow was subject to frequent interruptions (e.g., availability of ultracentrifuge and/or reagents etc.). During this period, ASs were deployed and wastewater was sampled at subcommunity or pooled subcommunity sewer maintenance holes, a node further downstream in the sewer-shed than would be used at later dates (Figure 1). The intention was to prioritize limited sampling capacity for dormitories or dormitory subcommunities (i.e., building wings) that were at a higher risk due to high occupancy (>100 persons). Wastewater from community sewer maintenance holes at both complexes 1 and 2 tested negative for the initial 10 days

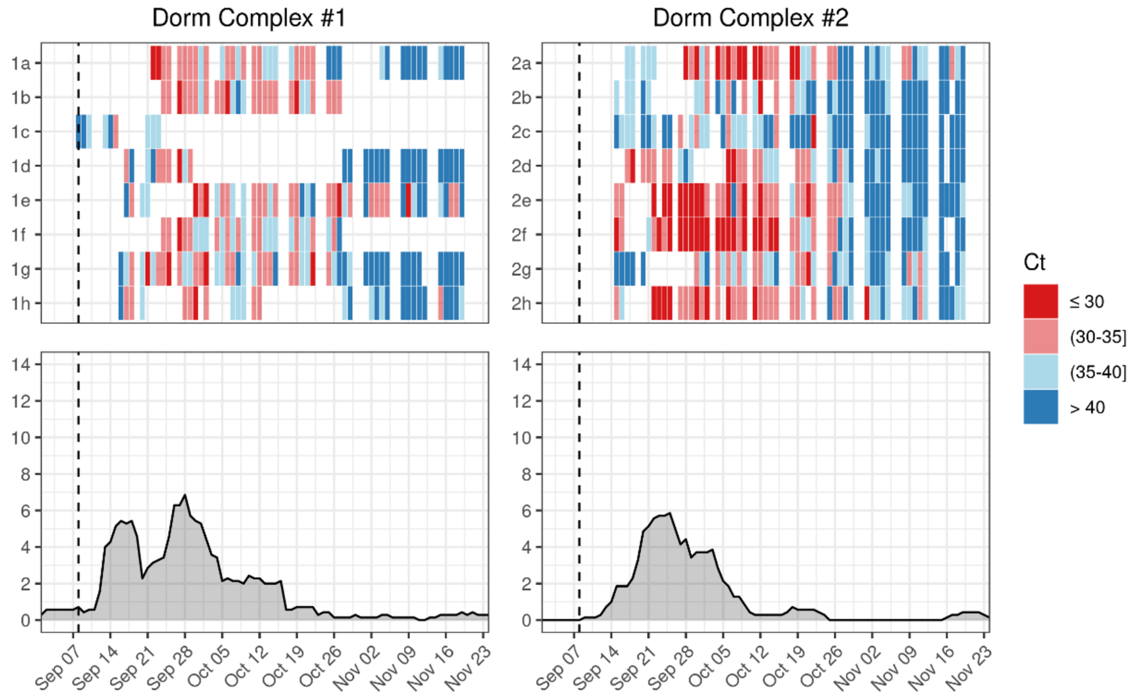

Figure 2. Wastewater positivity (Ct values) over time in fall 2020 obtained from SARS-CoV-2 RNA testing [top panel] and clinical case counts represented as a seven day moving average centered at the date (3 days before − 3 days after) [bottom panel]. Vertical dashed line represents the date of student move-in. Color-coded legend represents Ct-value categories.

of the fall 2020 semester, consistent with the requirement that all students obtain a negative test result before moving into university housing. By the third week of fall 2020 (September 16, 2020), all ASs were deployed at the building level, and unique building positive results were being used to inform testing of student occupants.

Early Warning Potential of Wastewater Surveillance during the Fall 2020. Starting early in the Fall 2020 semester, daily wastewater results from the prior 24 h period were made available for consideration by a multidisciplinary steering group at their daily 2:30 pm briefings. The wastewater data was used in conjunction with symptomatic testing early in the fall semester to make determinations about when point prevalence testing of occupants in a particular dormitory should be implemented (Figure S1). By the third week of September 2020, three dormitories had been subject to point prevalence testing on the basis of the co-occurrence of several symptomatic positives (based on symptomatic SHW cases) and persistent, strongly positive wastewater signals (Ct < 30 over two successive sampling days) (Figure 2 and Figure S2). These point prevalence testing events yielded eight, eight, and two asymptomatic/symptomatic cases for Dormitories 1a, 1d, and 2c, respectively. In an other instance, point prevalence testing was triggered solely on the basis of strongly positive wastewater testing results, revealing three positive asymptomatic/symptomatic cases in Dormitory 2f. In the following week, occupants in two more dormitories were subject to point prevalence testing on the basis of a combination of symptomatic clinical testing results and wastewater positivity. These testing events yielded 10 and 8 previously undetected cases, in Dormitories 2d and 2h, respectively.

By the last week of September, wastewater results from most of the sampled dormitories (11 of 13) were persistently, strongly positive, and it was decided that standing rolling point-prevalence testing for dormitory occupants was essential to prevent widespread outbreaks. This decision established a precedent whereby wastewater data should be used in conjunction with clinical diagnoses from symptomatic positives (via SHW testing), as well as reports of symptoms and exposures, as part of the decision-making process to prioritize which dormitories should undergo point prevalence testing each week. The largest number of positives from a single dormitory was 11 in a single two week period during the fall 2020.

Alignment of Wastewater Results with Clinical and Epidemiological Data. In fall 2020, all dormitory occupants had prearrival negative tests and wastewater samples were initially negative although positioning of the AS (wastewater sampling locations) and sampling was yet chaotic. SARS-CoV-2 positivity in wastewater began in the second week after the majority of students arrived and peaked in the fourth to fifth week after move-in and subsided by the eighth week. Thereafter wastewater results were mostly negative (Ct > 40) across all sampled sites with the exception of Dormitory 1e (Figure 2 and Figure S3). The trends in wastewater positivity were for the most part well-aligned with the clinical testing results. The maximum seven day moving average in fall 2020 was recorded to be six and seven cases in dormitory complex #1 and #2, respectively. During the peak period, corresponding wastewater samples from most dormitories gave consistent, strongly positive signals (Ct < 30). Overall, wastewater positivity was frequently detected before the occupant positivity in the corresponding dormitory and wastewater signal progressed with the number of positive occupants detected. Dormitories 1c and 2a were outliers for this trend between wastewater positive and case counts whereby cases were identified via clinical testing before corresponding building-level wastewater samples yielded positive results (Ct < 35).

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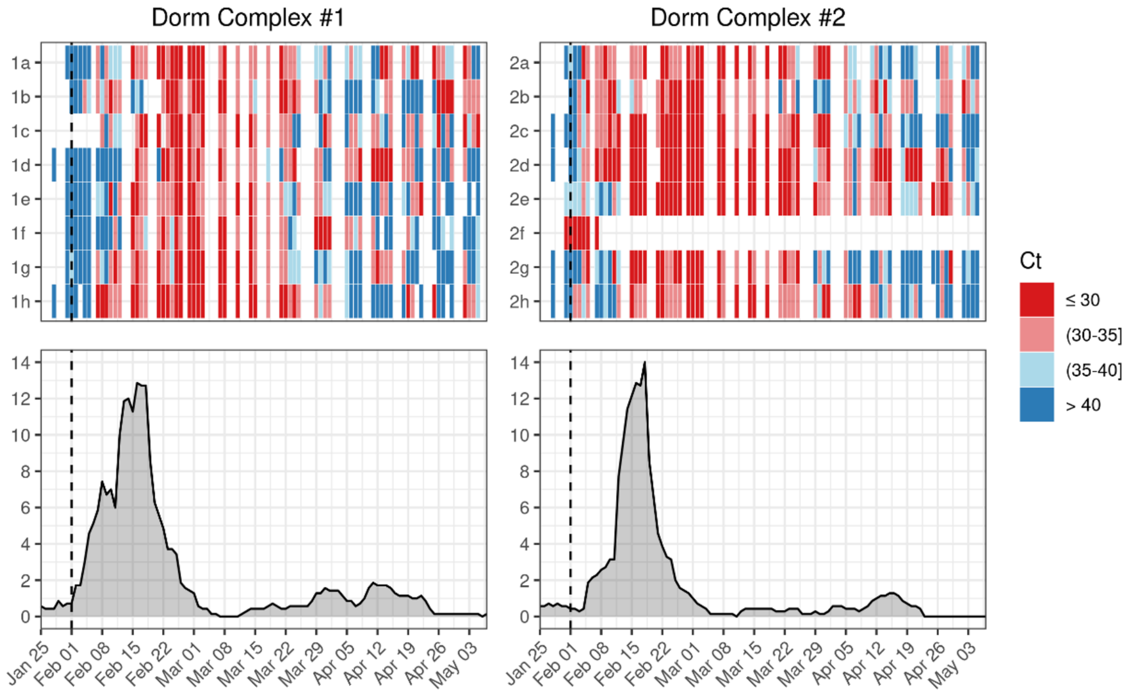

Figure 3. Wastewater positivity (Ct values) over time in spring 2021 obtained from SARS-CoV-2 RNA testing [top panel] and clinical case counts represented as seven day moving average centered at the date (3 days before − 3 days after) [bottom panel]. Vertical dashed line represents the date of student move-in. Color-coded legend represents Ct-value categories.

Wastewater sampling started much more smoothly in spring 2021 compared to that in fall 2020, because more ASs were available and relevant personnel were more familiar with the workflow. Wastewater positivity over time was similar to the start of fall 2020, whereby all dormitory occupants initially tested negative via clinical testing, and wastewater results from corresponding buildings were negative. However, a spike in wastewater positivity and positive cases counts was recorded early in the fall 2020 semester and occurred in the second week, peaking in the third−fourth weeks (Figure 3 and Figure S4). Generally, wastewater positivity was found to noticeably foreshadow the clinical cases detected in the second week in the dormitories. Unlike fall 2020, a trend in individual case counts in spring 2021 appeared to follow a unimodal peak with relatively higher number of cases per dormitory in the second−fourth week from students moving into the dormitories. At peak, the maximum seven day moving average was recorded to be 12 and 14 cases in dormitory complexes #1 and #, respectively. Also, unlike fall 2020, where individuals case counts subsided by the ninth week, individual case counts trickled through the 12 weeks of spring 2021. This was reflected in the wastewater positivity as well as with Ct value remaining <35 for a prolonged period of spring 2021. The overall trend in case-counts observed at both the dorm complexes sampled in fall and spring semesters closely aligned with the trend in cases recorded regionally (Figure S5).

Effect of Prolonged Convalescent Shedding on Wastewater Positivity. Retrospective analysis of data from fall 2020 reveals that there was good agreement between wastewater Ct score and the estimated number of individuals shedding SARS-CoV-2 RNA in the sampled dormitory building (Figure 4 and Figure S6). Wastewater positivity (Ct score) and the number of individuals shedding were prominent between the second and fifth weeks and were mostly negative after the ninth week. Dormitory 1e was an exception to this trend. Similar agreement was also observed for the spring 2021 data. However, as noted above, the number of positive cases detected during the peak period (second−fourth week after move-in) in spring 2021 was over 2-fold higher compared to a similar period during fall 2020 (Figure 2 and Figure 3). Between the fourth and eighth week period, while there were almost no new positive cases reported, the number of occupants shedding contributed to the wastewater positivity and this number included those returning to the respective dormitories following quarantine or isolation (Figure 5). As positive cases continued to trickle in for the remainder of spring 2021, the number shedding contributed to the prolonged wastewater positivity. Despite the number of newly infected occupants in Dormitories 1a and 2g being nearly zero in the latter half of spring 2021, a positive signal from the wastewater was consistently recorded. The overall correlation between wastewater Ct and number shedding in dormitories was deduced to be 0.389 and 0.470 for fall 2020 and spring 2021, respectively.

The shedding model used in this study (Figure S7) assumed that 50% of infected individuals shed into the wastewater.[18,19] On the basis of previously reported results,[20−22] our model specifies that, of the individuals who shed, a detectable rate of shedding will persist from the day of a positive test until 14 days postpositive test. The likelihood of detectable shedding then decreases rapidly for the next 10 days and continues to decrease, at a slower pace, until 50 days after the positive test, where we assume there is no longer a possibility of shedding. To accommodate individuals who may be shedding prior to a positive test date,[2] we assigned a small probability of shedding for up to 7 days before the positive test date.

DISCUSSION

Since the early days of the pandemic, there has been widespread interest in community-level surveillance, and

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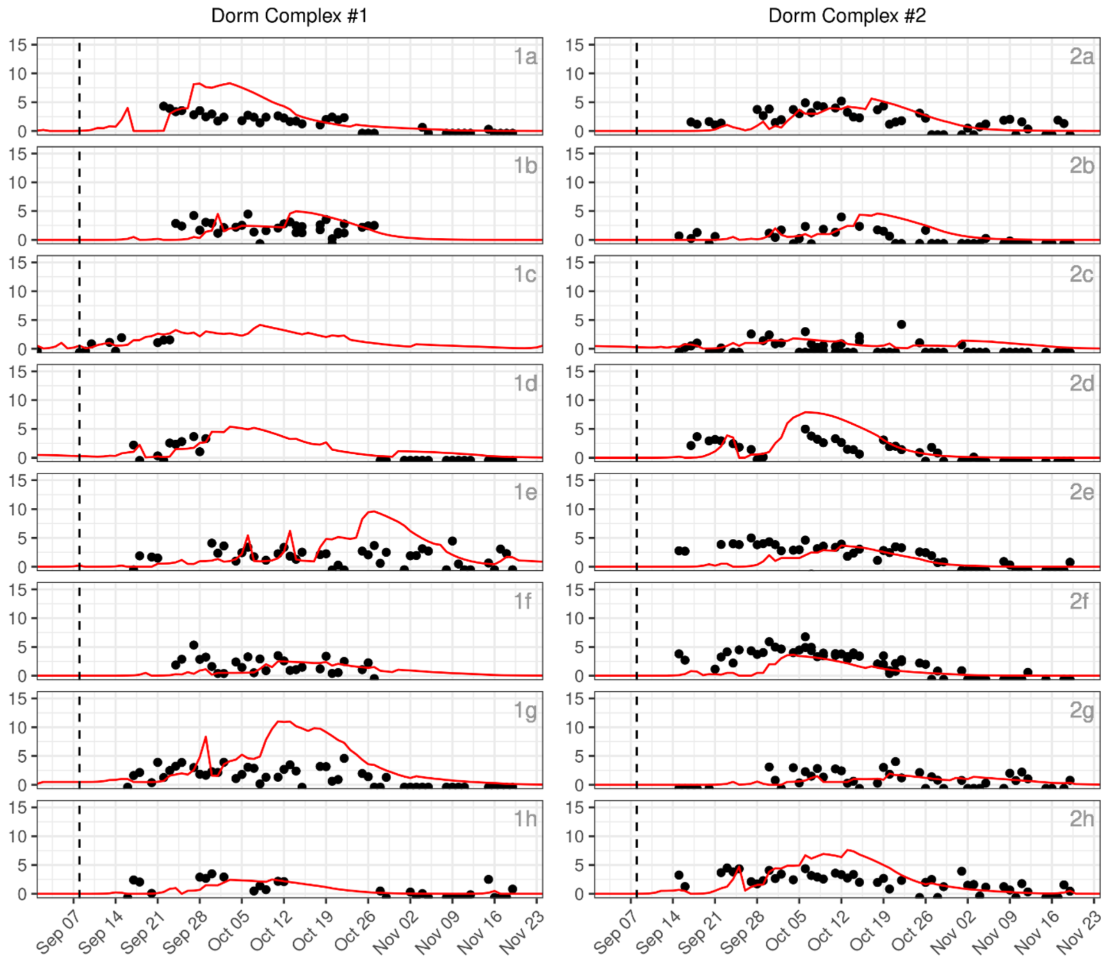

Figure 4. Estimated number of people shedding (red line) in each dormitory and the Ct score (●) across fall 2020. The Ct score is a rough estimate of the viral load in the wastewater. Vertical dashed lines represent the date of student move-in.

quick to follow was pooled building-level wastewater surveillance as a means to overcome logistic challenges associated with comprehensive testing in congregate settings. Wastewater surveillance is a valuable tool for COVID-19 outbreak detection particularly where routine and robust individual testing capabilities are limited. For pooled wastewater surveillance to be conclusive, it is important to realize that infection prevalence influences the target population size that can be pooled together in a single sample. When prevalence is low, it is reasonable to sample a larger population together in the same pool (e.g., wastewater combined from multiple dormitories or at subcommunity level). As the prevalence increased, it was helpful to reduce the population per sample and test wastewater at the building-level to obtain a better resolution. The final alternative, when prevalence is very high, is to systematically test all individuals in the building on a recurrent basis.

To be actionable, pooled wastewater surveillance results need to be available with very quick turn-around time, which can effectively inform the decision-making team and trigger point-prevalence testing (testing all targeted dormitory occupants). Less frequent wastewater surveillance (such as weekly) may not be sensitive enough to reliably detect new cases in residential buildings.[23] Other universities that performed wastewater surveillance shared a similar viewpoint.[9,11] Although 24 h composite wastewater samples were collected by the AS, subsequent transport of samples to the lab, lab workflow, and reporting/sharing of results typically required an additional 6−8 h depending on the number of samples processed. For this integrated process to occur successfully on a near daily basis over a one year period required a streamlined effort with a large well-coordinated team working across many disciplines.

While daily frequency of wastewater testing was largely helpful to prevent large outbreaks, the volume of raw data on wastewater positivity collected was tedious and challenging to interpret on a real-time basis. Often for a judicious response, it was necessary to look for trends in wastewater positivity over several successive days rather than relying on a single positive result following AS deployment and wastewater testing. For example, triggering point-prevalence testing in the dormitory based on a positive wastewater signal was often in the context of several other factors or relocation and timing of limited AS available to a dormitory building that was at higher risk (e.g., as occurred during shortage of AS in early fall 2020). Wastewater surveillance was more effective as an early warning tool when prevalence was low in the dormitory occupants, which made it easier to detect new cases and enforce isolation and quarantine measures. In contrast, when community prevalence was high, pooled wastewater-surveillance became inconclusive on its own, and it was especially difficult to distinguish whether wastewater positivity was indicative of new cases or due to returned convalescent shedders. This may have compounded the challenges in interpreting and responding with resources to

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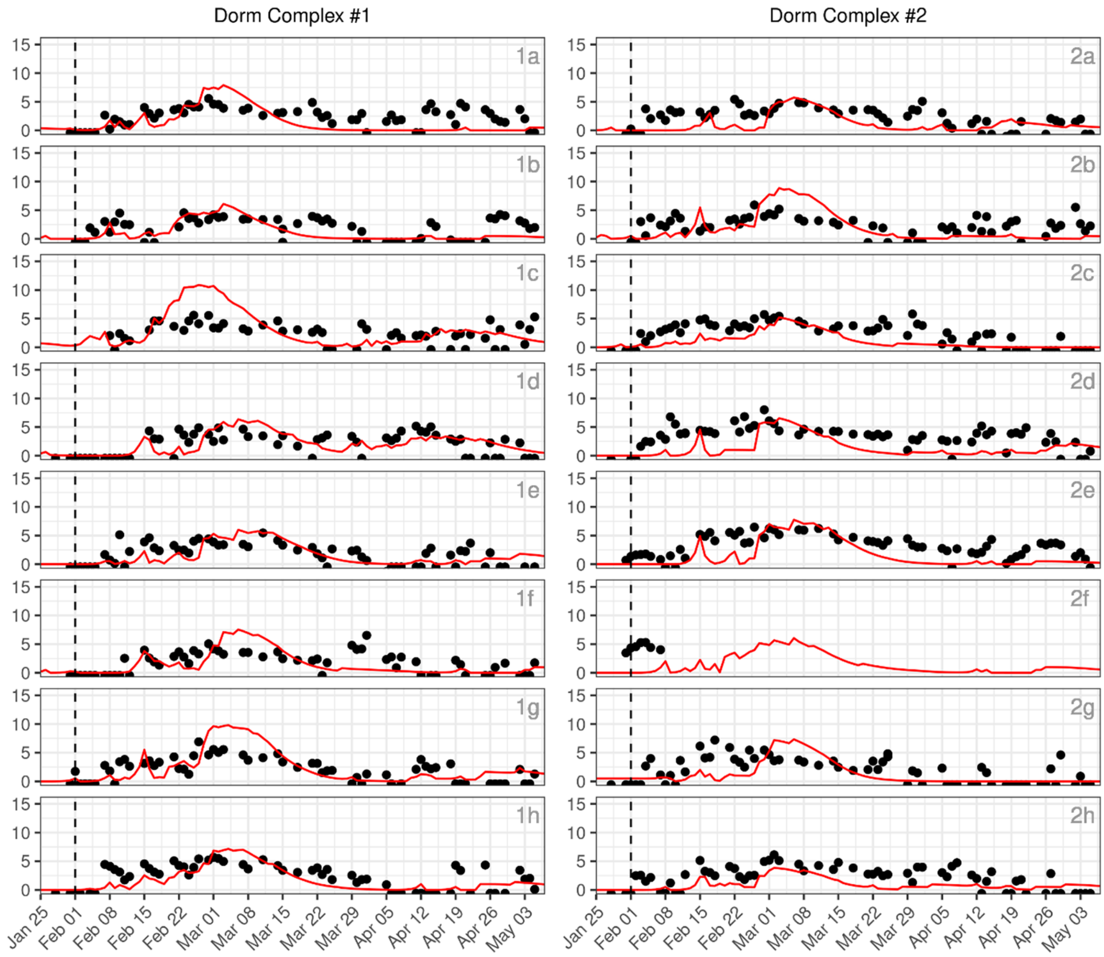

Figure 5. Estimated number of people shedding (red line) in each dormitory and the Ct score (●) across spring 2021. The Ct score is a rough estimate of the viral load in the wastewater. Vertical dashed lines represent the date of student move-in.

the data in real-time. To our knowledge, our wastewater monitoring program employed much more frequent, robust, and consistent building-level wastewater sampling compared to other universities in the US.[9]

Challenges in Interpretation of Wastewater Positivity. Prolonged wastewater positivity (Ct < 35) was sometimes observed even when the estimated number of occupants shedding in a corresponding dormitory was zero. These circumstances were seen on a few occasions in fall 2020 (Dormitories 2a and 2h) and in almost all dormitories tested in spring 2021. This phenomenon can be attributed to convalescent shedding from those previously infected residents that returned to their dormitory following the conclusion of their isolation period.[9,10,21] It is well-documented that the period of RNA shedding in the feces is longer than respiratory modes,[24] and a median fecal shedding time of 25 days was reported among hospitalized COVID-19 patients.[21] Our model accounts for persistent shedding after the end of the infectious period by assigning 0.25 probability of shedding for the 25 day period after a positive test result (Figure S4).

University housing situations fluctuated throughout the year but dormitory rosters only captured one point in time (initial move-ins). A few scenarios that were challenging to track include: (i) some dormitory occupants left campus after a positive test (isolation at home) and did not return (did remote learning or withdrew), (ii) a small fraction of dormitory residents may have moved off-grounds at some point during the semester, (iii) a fraction of dormitory residents that may not have used the toilets in their respective dormitories, and (iv) staff and visitors using the toilets in the dormitories that may also have contributed to wastewater positivity. While over 80% compliance was observed for clinical surveillance testing and isolation and quarantine adherence, noncompliant residents or nonresidents could have contributed to wastewater positivity.

In the case of Dormitory 2f, high baseline wastewater positive signal at the start of spring 2021 and subsequent positive cases were detected. Accordingly, it was challenging to differentiate new cases versus convalescent shedding via wastewater sampling from that building. It was therefore decided to suspend AS deployment at this location for the remainder of the semester and deploy the AS at another higher risk location where detection might result in a more effective public health response. The collected wastewater at Dormitory 2f frequently had unusually "stooly" and undiluted appearance on a regularly basis. The cause for this phenomenon was not further pursued, but it was presumed that sample water quality could have influenced Ct measurements. The retention of SARS-CoV-2 RNA in simulated wastewater biofilms has been documented;[25] however, further investigation may be necessary. From a quantitation standpoint, it was challenging to normalize results using the molecular methods employed in this study, the development of a mathematical model was therefore critical to this work.

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Challenges in Wastewater Sampling and Testing. While building-level wastewater surveillance is useful for real-time decision making, it is highly resource and effort intensive (i.e., it requires many AS and significant personnel time to collect and process the samples). Severe supply shortage of AS compelled the need for construction and deployment of homemade AS in fall 2020. Other universities used different strategies to overcome this challenge, e.g., collecting WW grab samples instead of overnight composites and/or using alternative sample collection methods such as passive samplers.[26,27] Passive samplers are cost-effective and require fewer resources to deploy and process; therefore, they may be especially promising for long-term monitoring efforts in resource-constrained settings.

Safely accessing wastewater from target sampling locations can be a challenge. Construction in few of the dormitory buildings were such that they lacked an exclusive sewer maintenance hole or cleanout valve that could allow for the collection of a representative wastewater discharge; therefore, building-level surveillance for such dormitories was not possible. Similarly conjoint building design with multiple discharge sewer maintenance holes, shared bathrooms, and untraceable movement of occupants within the building can make wastewater sampling and attribution to a group of occupants challenging. Most dormitory buildings sampled in the study had >100 residents contributing to the wastewater; buildings with <20 occupants at a given time were often associated with low wastewater flow at the sewer maintenance hole, leading to challenges in wastewater sample collection in the AS and subsequent processing. Fluctuations in wastewater flow rate and marked variability in sample collection volumes in AS was also observed. Although rare, it sometimes happened that a particular AS would not collect any sample overnight (e.g., due to clogging of the intake, misalignment of the sample probe in the wastewater stream, etc.). These occurrences were mostly clustered at the beginning of fall 2020, when methods were not yet optimized and personnel had not had much experience using the AS. This was documented as a challenge by other universities and institutions that used wastewater surveillance for SARS-CoV-2.[9]

Extreme weather conditions can influence wastewater sample collection as all wastewater sewer maintenance hole at the collection sites were outdoor for AS deployment. For periods when daytime temperatures exceeded 38 °C, the ice packed around the sample collection jar in the AS was found to melt rapidly, potentially contributing to SAR-CoV-2 RNA degradation prior to the sample processing. Conversely, temperatures below 0 °C resulted in frozen wastewater within the AS collection tubing and/or the sample collection jar, and in some instances, no collection was also recorded.

There were also some instances in which building occupants purposely interfered with the wastewater sampling equipment. During fall 2020, sporadic instances of interruption in wastewater collection due to noncompliance and resistance from dormitory residents was recorded. AS and/or barricades around AS were vandalized on multiple instances that required counter measures to ensure uninterrupted wastewater sample collection. Noncompliance with regard to dormitory residents not using the toilets in the building was reported on a few occasions. University-wide education on wastewater testing and awareness initiatives were put in place to counter these instances of occupant behaviors.

As highlighted earlier,[9] in order for wastewater surveillance to be effective, it requires a proactive, cross-disciplinary, collaborative effort including, clinical, engineering, data-science, student health, and maintenance/facilities professionals. Building-level pooled wastewater surveillance at universities during a pandemic was a valuable tool as it provided high-resolution spatial sampling in a well-controlled context that was passive to the individual occupants. For universities to remain open/reopen during a pandemic it will be necessary to develop practical and effective ways of living with SARS-CoV-2. Although vaccination is expected to provide additional individual protection, it will take time and great efforts to reach high population coverage and reduce virus morbidity and transmission in many settings. Implementation and evaluation of tailored testing, contact tracing, and isolation need to continue, so that schools and universities can preserve students, staff, and community safety.

CONCLUSIONS

Wastewater surveillance is a powerful public health forecasting tool, and its attractiveness is in its conceptual simplicity. Findings from this one year case-study highlight the effectiveness of building-level wastewater surveillance by providing high-resolution passive sampling, is in proactively identifying hotspots, and informing the decision-making process for triggering point-prevalence in individual dormitories. UVA's wastewater monitoring program employed high frequency and widespread sampling and was potentially unique in the extent to which it was integrated with other health data streams to facilitate decision-making. Wastewater surveillance serves as a true early warning system only when prevalence is low and/or clinical testing of the surveilled population is scarce or deficient. When prevalence is very high, it is more beneficial to systematically test all individuals in the building on a recurrent basis.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestwater.2c00057.

Figures of cross-functional workflow of wastewater surveillance, three different makes of autosamplers used in the study for composite wastewater collection, actual case counts and average wastewater Ct values recorded for each dormitory, trend in recorded regional case-counts overlaid on the clinical case counts, boxplots showing the distribution of the estimated number shedding as a function of Ct value, and model depicting probability of shedding over time from a positive test result (PDF)

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Notes

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